Exhibit A

29

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF MICHIGAN
SOUTHERN DIVISION

SUN PHARMACEUTICAL INDUSTRIES LTD.,

Plaintiff,

v.

ELI LILLY AND COMPANY,

Defendant.
_____/

Case: 2:07-cv-15087
Judge: Steeh, George Caram
Referral MJ: Whalen, R. Steven
Filed: 11-29-2007 At 04:12 PM
CMP SUN PHARMACEUTICAL V ELI LILLY AND COMPANY (RRH)

COMPLAINT

Plaintiff Sun Pharmaceutical Industries Ltd. ("Sun"), by its attorneys, for its Complaint against Eli Lilly and Company ("Defendant"), alleges as follows:

INTRODUCTION

1. Sun brings this action for declaratory judgment of patent invalidity and non-infringement under the federal Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, and 21 U.S.C. § 355(j)(5)(C)(i), which is part of the Hatch-Waxman Amendments to the Federal Food, Drug, and Cosmetic Act ("FFDCA"), as amended by Title XI of the Medicare Prescription Drug, Improvement, and Modernization Act of 2003, Pub. L. No. 108-173, 117 Stat. 2066 (2003) ("MMA").

2. This action arises out of Sun's submission of an Abbreviated New Drug Application ("ANDA") to the U.S. Food and Drug Administration ("FDA") seeking approval to

market a generic version of Defendant's brand-name cancer medication Gemzar®, known generically as gemcitabine.

3. Defendant purports to own U.S. Patent No. 5,464,826 ("the '826 patent"). Upon submission by Defendant, the '826 patent was listed in FDA's compilation of approved drugs and their respective patents entitled "Approved Drug Products With Therapeutic Equivalence Evaluations," commonly referred to as the "Orange Book." As a consequence of such Orange Book listing, Defendant maintains, and has affirmatively represented to the world, that the '826 patent claims the approved drug Gemzar®, or a method of using that drug, and that a claim for patent infringement could reasonably be asserted against any generic ANDA applicant, including Sun, attempting to market a generic gemcitabine product before expiration of the '826 patent.

4. Sun seeks to market a generic gemcitabine product before the expiration of the '826 patent. Therefore, as required by the FFDCA, Sun has certified to the FDA that its ANDA products will not infringe any valid or enforceable claims of the '826 patent and has further notified Defendant of the legal and factual bases for those certifications. Sun's submission of this so-called paragraph IV certification to the '826 patent constitutes an artificial act of patent infringement, putting Sun at considerable risk of being sued by Defendant both before and after market entry.

5. This regulatory submission creates the necessary case or controversy and subject matter jurisdiction for Defendant to sue Sun – and for Sun to obtain declaratory judgment against Defendant – regarding infringement of the '826 patent.

6. In a separate lawsuit, *Eli Lilly and Company v. Sun Pharmaceutical Industries Ltd.*, Southern District of Indiana Case No. 1:06-cv-1721-SEB-VSS, Defendant, in fact, did sue

Sun for infringement of the '826 patent. Sun was not subject to personal jurisdiction in the Southern District of Indiana, and Defendant voluntarily dismissed that case without prejudice on or about September 4, 2007.

7. In filing the Indiana lawsuit, however, Defendant triggered, under 21 U.S.C. § 355(j)(5)(B)(iii), a 30-month stay during which the FDA may not approve Sun's ANDA.

8. Sun has satisfied all substantive requirements for approval of its ANDA and is prepared to begin commercial marketing of its competing generic product before expiration of the '826 patent. But Sun's approval is currently being blocked by the listing of that patent in the FDA's "Orange Book" – an obstacle that can be removed by a judicial finding of invalidity, non-infringement, and/or unenforceability.

9. Sun faces potentially enormous infringement liability if it markets its generic product prior to expiration of the '826 patent. Only a declaratory judgment from this Court can alleviate this harm and allow Sun to obtain approval of its product and compete in the gemcitabine market free from such potential liability.

10. Accordingly, there is an actual, substantial, and continuing justiciable case and controversy between Sun and Defendant regarding the '826 patent, over which this Court can and should exercise jurisdiction and declare the rights of the parties.

11. Sun is entitled to a judicial declaration that the manufacture, sale, offer for sale, use, or importation of Sun's proposed generic gemcitabine product does not and will not infringe the '826 patent, and/or that the '826 patent is invalid. Absent the exercise of jurisdiction by this Court and such declaratory relief, Sun and the American public will be irreparably harmed by the substantial delay in the market entry and availability of lower-priced generic gemcitabine.

THE PARTIES

12. Plaintiff Sun is an Indian corporation having a principal place of business at Acme Plaza, Kurla Road, Andheri (E), Mumbai 400 059. Sun is in the business of developing and marketing generic pharmaceuticals. Sun's wholly owned United States subsidiary, Caraco Pharmaceutical Laboratories, Ltd., is a Michigan corporation have a principal place of business at 1140 Elijah McCoy Drive, Detroit, Michigan 48202.

13. On information and belief, Defendant Eli Lilly is an Indiana corporation, having its corporate offices and principal place of business at Lilly Corporate Center, Indianapolis, Indiana 46285.

U.S. PATENT NO. 5,464,826

14. On information and belief, the '826 patent, titled "Method of Treating Tumors in Mammals with 2',2'-Difluoronucleosides" was issued to Defendant by the United States Patent and Trademark Office on November 7, 1995. A true and correct copy of the '826 patent is attached as Exhibit A. On information and belief, Defendant has been the owner of the '826 patent since it issued.

15. On information and belief, the '826 patent is scheduled to expire on November 7, 2012, followed by a six-month period of market exclusivity granted by the FDA under 21 U.S.C. § 355(a), ending on May 7, 2013.

16. On information and belief, Defendant has the right to sue for any infringement of the '826 patent.

JURISDICTION AND VENUE

17. Substantial, present, genuine, and justiciable controversies exist between Defendant and Sun regarding the '826 patent.

18. This action arises under the Patent Laws of the United States, 35 U.S.C. §§ 1 *et seq.*; and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

19. This Court has original jurisdiction over the subject matter of this action under 28 U.S.C. §§ 1331 and 1338(a), because it involves substantial claims arising under the United States Patent Act, 35 U.S.C. §§ 1 *et seq.*; and under the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, because it is an actual controversy concerning the '826 patent.

20. This Court can and should declare the rights and legal relations of the parties regarding the '826 patent pursuant to the Declaratory Judgment Act, 28 U.S.C. §§ 2201, 2202.

21. On information and belief, this Court has personal jurisdiction over Defendant because Defendant conducts substantial business in, and has regular and systematic contact with, this District.

22. On information and belief, Defendant maintains such a continuous and systematic contact with the State of Michigan and this District by conducting substantial, regular, and systematic business therein through the marketing and sales of their pharmaceutical products, including Gemzar® – the purported commercial embodiment of the '826 patent – to allow this Court to reasonably exercise personal jurisdiction over Defendant.

23. Upon information and belief, Defendant purposefully avails itself of the privilege of doing business in the State of Michigan and in this District.

24. Venue is proper in this judicial district under 28 U.S.C. §§ 1391(b) and 1391(c).

BACKGROUND

I. Regulatory Framework

A. FDA Approval Of New Drug Applications (NDAs)

25. The FFDCA, 21 U.S.C. § 301 *et seq.*, as amended by the Hatch-Waxman Amendments and Title XI of the MMA, sets forth the rules that the FDA follows when considering whether to approve the marketing of both brand-name and generic drugs.

26. Under the FFDCA, an applicant seeking to market a new brand-name drug must prepare an NDA for consideration by FDA. *See* 21 U.S.C. § 355.

27. The NDA must include, among other things, the number of any patent that claims the "drug" or a "method of using [the] drug" for which the NDA was submitted and for which a claim of patent infringement could reasonably be asserted against an unauthorized party. *See* 21 U.S.C. § 355(b)(1), -(c)(2); 21 C.F.R. § 314.53(b), -(c)(2).

28. Upon approval of the NDA, FDA publishes patent information for the approved drug in the Orange Book. *See* 21 U.S.C. § 355(j)(7)(A)(iii).

29. By filing an NDA and submitting a patent for listing in the Orange Book, the NDA-holder/patent owner, by law, necessarily maintains that the listed patent claims the approved NDA drug, or a method of using that drug, and that an infringement suit could reasonably be asserted against anyone who engages in the manufacture, use, or sale of the drug, and, in particular, against any company that is seeking to make a generic bioequivalent of the NDA drug before patent expiration.

30. Thus, the NDA-holder/patent owner necessarily puts all prospective generic ANDA applicants on notice that a suit for infringement can and will be asserted against any

ANDA applicant that attempts to seek approval for and market a generic version of the NDA drug before patent expiration.

31. Such conduct by the NDA-holder/patent owner gives rise to a real and concrete belief on the generic applicant's part that it will face an infringement suit, or the threat of one, if it attempts to seek approval for or to market a generic version of the NDA drug before patent expiration.

B. Generic Competition – Abbreviated New Drug Applications (ANDA)

32. Generic drugs are versions of brand-name prescription drugs that typically contain the same active ingredients, but not necessarily the same inactive ingredients, as the brand-name original.

33. In 1984, Congress enacted the Drug Price Competition and Patent Term Restoration Act, also known as the Hatch-Waxman Amendments to the FFDCA. *See* Pub. L. No. 98-417, 98 Stat. 1585 (1984) (codified at 21 U.S.C. § 355 and 35 U.S.C. §§ 156, 271(e)).

34. Congress passed the Hatch-Waxman Amendments, which simplified the procedure for obtaining approval of generic drugs, for the purpose of decreasing the cost of pharmaceuticals through increased competition and to expedite the marketing of lower-priced generic drug products. Under the Hatch-Waxman Amendments, a generic manufacturer submits what is called an ANDA.

35. To receive approval of its ANDA, an applicant must show, among other things, that its generic drug is "bioequivalent" to the listed reference drug. *See* 21 U.S.C. § 355(j)(4)(F).

36. An ANDA also must contain a "certification" to each patent that the NDA holder has submitted to FDA for listing in the Orange Book in connection with the listed reference drug. *See* 21 U.S.C. § 355(j)(2)(A)(vii); 21 C.F.R. § 314.94(a)(12).

37. A so-called "paragraph IV" certification asserts that the listed patent is invalid, unenforceable, and/or will not be infringed and, on that basis, seeks FDA approval of the generic product before patent expiration. *See* 21 U.S.C. § 355(j)(2)(A)(vii)(IV); 21 C.F.R. § 314.94(a)(12). The submission of a paragraph IV certification has two important consequences.

38. First, a generic applicant that is first to submit an ANDA containing a paragraph IV certification for a listed patent is entitled to 180 days of generic market exclusivity during which no other competing generic drug products may be marketed. 21 U.S.C. § 355(j)(5)(B)(iv). This statutory benefit to the first filer is commonly known as "180-day exclusivity."

39. In particular, the statutory provision of the FFDCA applicable here provides that "[i]f the application contains a certification described in subclause (IV) of paragraph (2)(A)(vii) and is for a drug for which a previous application has been submitted under this section [containing] such a certification, the application shall be made effective not earlier than one hundred and eighty days after" the earlier of: (a) the first commercial marketing of that ANDA applicant's proposed drug; or, (b) a court decision – whether it involves the first applicant or not – that the particular patent that is the subject of the paragraph IV certification is invalid or not infringed. 21 U.S.C. § 355(j)(5)(B)(iv). Thus, unless a subsequent generic applicant can obtain a court decision of noninfringement and/or invalidity as Congress intended, the approval of its ANDA can be delayed indefinitely by the purported exclusivity of the first-filer.

40. Second, the submission of a paragraph IV certification for a listed patent constitutes an artificial act of infringement that creates the necessary case or controversy and subject matter jurisdiction to enable an NDA-holder/patent owner to file, and a district court to resolve, an action for patent infringement – before the generic drug is actually made, used, or sold – to determine whether the generic drug, if marketed and sold in accordance with the ANDA, would infringe the relevant patent.

41. An applicant submitting an ANDA containing a paragraph IV certification must notify both the NDA holder and patent owner of its paragraph IV certification. *See* 21 U.S.C. § 355(j)(2)(B)(i).

42. Upon receiving notice of the paragraph IV certification, the NDA holder/patent owner has 45 days in which to file an infringement suit against the generic manufacturer. *See* 21 U.S.C. § 355(j)(5)(B)(iii); 35 U.S.C. § 271(e)(2)(A).

43. The NDA holder/patent owner's filing of a lawsuit prior to the expiration of 45 days prevents FDA from issuing final approval of the generic maker's ANDA for a period of 30 months, absent certain exceptions. *See* 21 U.S.C. § 355(j)(5)(B)(iii).

II. Sun's ANDA No. 78-433

44. Defendant listed the '826 patent in the Orange Book in connection with NDA No. 20-509 and the brand name drug Gemzar®, which comprises the active ingredient gemcitabine.

45. By listing the '826 patent in the Orange Book, Defendant maintains, and has affirmatively represented to the world, that the '826 patent claims Gemzar®, or a method of using that drug, and that an infringement suit could reasonably be asserted against any generic

ANDA applicant, including Sun, that attempts to seek approval for, and market, a generic version of Gemzar® before the expiration of the '826 patent. The listing of the '826 patent in the Orange Book alone objectively creates the necessary case or controversy and subject matter jurisdiction for an ANDA-filer who makes a paragraph IV certification as to the '826 patent.

46. On July 28, 2006, Sun filed an ANDA (No. 78-433) with the FDA seeking generic approval for the commercial manufacture, use, sale, and/or importation of Gemcitabine for Injection, 200 mg base/vial and 1g base/vial (the "ANDA Products"), generic versions of Defendant's Gemzar®.

47. Because Sun seeks FDA approval to market its ANDA Products before expiration of the '826 patent, Sun's ANDA includes paragraph IV certifications to the '826 patent.

48. On October 17, 2006, Sun sent to Defendant a statutorily required notice letter of its paragraph IV certifications, which contains a detailed factual and legal statement as to why the '826 patent is invalid, unenforceable, and/or not infringed by Sun's ANDA Products.

49. On information and belief, Defendant received Sun's notice letter of its paragraph IV certifications on October 20, 2006.

50. On December 1, 2006, Defendant filed a patent infringement lawsuit against Sun, alleging that Sun's ANDA Products would infringe the '826 patent. Defendant voluntarily dismissed that lawsuit without prejudice on September 4, 2007.

51. Sun seeks certainty as to infringement, validity, and enforceability of the '826 patent as it moves forward with its efforts to develop and market its proposed gemcitabine formulation in the United States.

52. Infringement in the United States under 35 U.S.C. § 271(e)(2)(A) for the act of submitting an ANDA to the FDA has been characterized as a "highly artificial act of infringement," *Eli Lilly & Co. v. Medtronic, Inc.*, 496 U.S. 661, 678 (1990), where the actual manufacture, use or sale of a drug in violation of a patent has not occurred, but is recognized to allow parties to seek judicial adjudication of their rights, and to prevent uncertainty.

53. Until and unless Sun obtains a court decision of noninfringement and/or invalidity on the '826 patent, it faces potentially enormous infringement liability if it commences marketing before the '826 patent expires. Sun can alleviate this harm and obtain patent certainty only through a declaratory judgment from this Court on the '826 patent.

COUNT I
DECLARATION OF INVALIDITY AND NON-INFRINGEMENT OF THE '826 PATENT

54. Sun realleges and incorporates by reference the allegations of Paragraphs 1-53.

55. A conflict of asserted rights has arisen and a justiciable controversy exists between Sun and Defendant with regard to the infringement, validity, and enforceability of the '826 patent, including whether the manufacture, use, or sale of Sun's ANDA Products would infringe one or more claims of the '826 patent.

56. Sun has not infringed, contributed to the infringement of, or induced the infringement of any valid claim of the '826 patent and is not liable for infringement thereof.

57. The '826 patent is invalid, void and/or unenforceable against Sun, at least, for failure to comply with the requirements of at least 35 U.S.C. §§ 101, 102, 103 and/or 112.

58. Sun is entitled to declaratory judgment adjudicating its rights with respect to the '826 patent.

Prayer for Relief

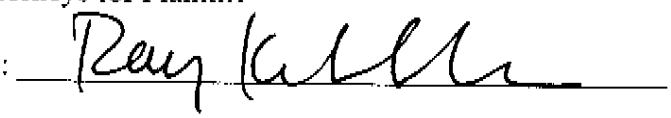
WHEREFORE, Plaintiff Sun Pharmaceutical Industries Ltd. respectfully requests that this Court enter a Judgment and Order in its favor and against Defendant as follows:

(a) declaring that Sun has not infringed and that Sun's manufacture, use, or sale of products covered by ANDA No. 78-433 would not infringe the claims of U.S. Patent No. 5,464,826;

(b) declaring that each claim of the '826 patent is invalid and/or unenforceable; and

(c) granting Sun any further and additional relief as the Court deems just, proper and equitable under the circumstances.

BUSH SEYFERTH KETHLEDGE & PAIGE, PLLC
Attorneys for Plaintiff

By: 

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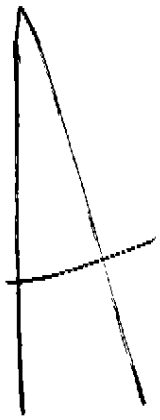

US005464826A

United States Patent [19]

Grindey et al.

[11] Patent Number: 5,464,826

[45] Date of Patent: Nov. 7, 1995

[54] **METHOD OF TREATING TUMORS IN MAMMALS WITH 2',2'-DIFLUORONUCLEOSIDES**

[75] Inventors: **Gerald B. Grindey; Larry W. Hertel**, both of Indianapolis, Ind.

[73] Assignee: **Eli Lilly and Company**, Indianapolis, Ind.

[21] Appl. No.: **280,687**

[22] Filed: **Jul. 26, 1994**

Related U.S. Application Data

[62] Division of Ser. No. 99,268, Jul. 29, 1993, abandoned, which is a continuation of Ser. No. 746,441, Aug. 16, 1991, abandoned, which is a division of Ser. No. 163,571, Mar. 3, 1988, Pat. No. 5,061,793, which is a continuation of Ser. No. 786,419, Oct. 10, 1985, abandoned, which is a continuation-in-part of Ser. No. 677,783, Dec. 4, 1984, abandoned.

[51] **Int. Cl.**[6] **A61K 31/70**; C07H 19/073

[52] **U.S. Cl.** **514/50**; 514/49

[58] **Field of Search** 514/49, 50

[56] **References Cited**

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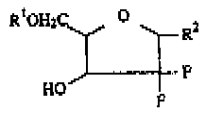
Primary Examiner—Douglas W. Robinson
Assistant Examiner—Gary L. Kunz
Attorney, Agent, or Firm—Robert A. Conrad

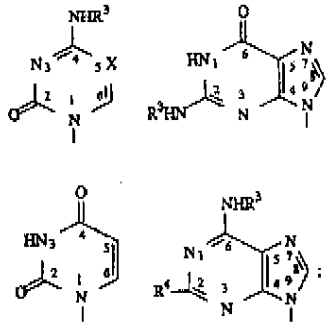
[57] **ABSTRACT**

A method of treating susceptible neoplasms in mammals comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the formula



I

wherein:
R^1 is hydrogen;
R^2 is a base defined by one of the formulae



X is C–R^4;
R^3 is hydrogen;
R^4 is hydrogen, C_1–C_4 alkyl, bromo, fluoro, chloro or iodo;
and the pharmaceutically-acceptable salts thereof.

7 Claims, No Drawings

METHOD OF TREATING TUMORS IN MAMMALS WITH 2',2'-DIFLUORONUCLEOSIDES

CROSS REFERENCE TO RELATED APPLICATION

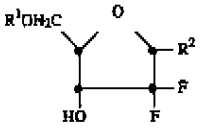
This application is a division of application Ser. No. 08/099,268, filed Jul. 29, 1993, now abandoned, which is a continuation of application Ser. No 07/746,441, filed Aug. 16, 1991, now abandoned, which is a division of application Ser. No. 07/163,571, filed Mar. 3, 1988, now U.S. Pat. No. 5,061,793, which is a continuation of application Ser. No. 06/786,419, filed Oct. 10. 1985, now abandoned, which is a continuation-in-part of application Ser. No. 06/677,783, filed Dec. 4, 1984, now abandoned.

BACKGROUND OF THE INVENTION

While the treatment of cancer was once considered impossible, great strides have been made during the past ten years in controlling the ravages of this often fatal disease. Several drugs which contribute to the increasing rate of survival are now routinely used clinically. The most commonly employed antitumor agents include methotrexate, doxorubicin and the vinca alkaloids such as vincristine. However, research continues to develop more effective compounds with greater safety for subjects under treatment. This invention provides an additional method of treating tumors.

SUMMARY OF THE INVENTION

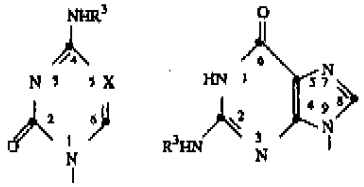
The present invention provides a method of treating susceptible neoplasms in mammals comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the formula



wherein:

R^1 is hydrogen or

R^2 is a base defined by one of the formulae



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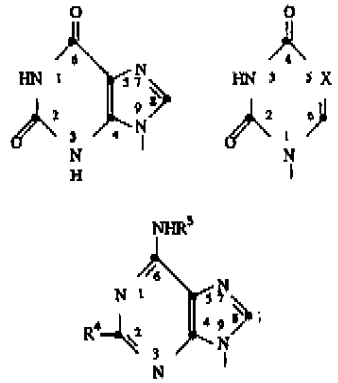


X is N or C–R^4;

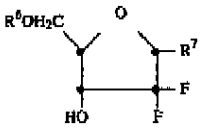
R^3 is hydrogen, C_1–C_4 alkyl or

R^4 is hydrogen, C_1–C_4 alkyl, amino, bromo, fluoro, chloro or iodo;

each R^5 independently is hydrogen or C_1–C_4 alkyl; and

the pharmaceutically-acceptable salts thereof.

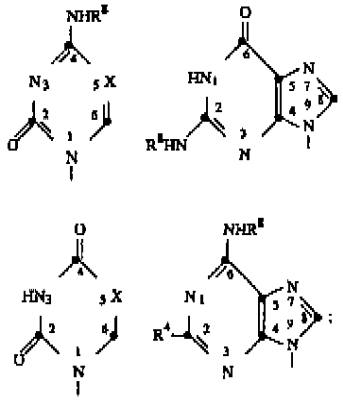
The present invention also provides a novel compound of the formula



wherein:

R^6 is hydrogen or C_1–C_4 alkyl;

R^7 is a base of one of the formulae



X is N or C–R^4;

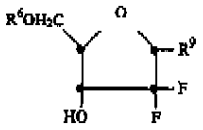
R^5 is hydrogen or C_1–C_4 alkyl;

R^4 is hydrogen, C_1–C_4 alkyl, amino, bromo, fluoro,

chloro and iodo; and

the pharmaceutically-acceptable salts thereof; with the proviso that R^6 and R^8 may both be hydrogen only when X is N.

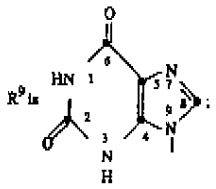
The present invention further provides a compound of the formula



III

wherein:

R^6 is hydrogen or C_1–C_4 alkyl;



and

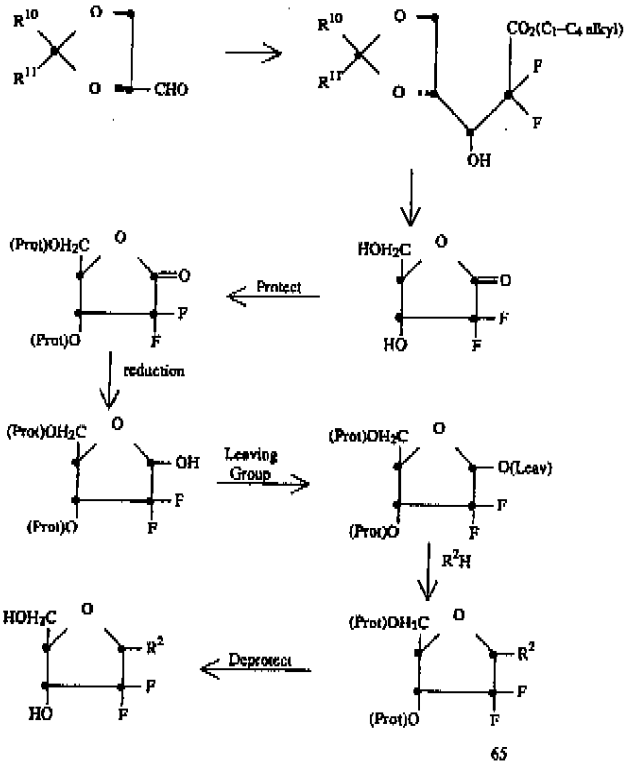
the pharmaceutically-acceptable salts thereof.

The present invention also provides pharmaceutical formulations useful for treating susceptible neoplasms in mammals comprising a compound of formula I with a suitable

pharmaceutically acceptable carrier, diluent or excipient therefor.

DETAILED DESCRIPTION OF THE INVENTION

The compounds employed in the present invention are preferably prepared by reacting a D-glyceraldehyde ketonide with a C_1–C_4 alkyl bromodifluoroacetate to afford an alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate. The hydroxypropionate is hydrolyzed to a lactone which is protected and reduced to afford a 2-deoxy-2,2-difluororibose or xylose derivative. The hydroxy group of this compound is provided with a leaving group, and the resulting carbohydrate is coupled with an appropriate base. The resulting protected nucleoside is finally deprotected to provide a compound for use in the present method. The overall reaction scheme is illustrated as follows:

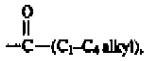


wherein R^{10} and R^{11} independently are C_1-C_3 alkyl,

"Prot" is a hydroxy protecting group and "Leav" is a leaving group.

It generally is desirable to convert free hydroxy groups to protected hydroxy groups during coupling of the 2-desoxy-2,2-difluorocarbohydrate to a base. The protecting groups are those commonly used in synthetic organic chemistry. Chemists are accustomed to choosing groups which can be placed efficiently on hydroxy groups, and which can be removed easily when the reaction is complete. Suitable groups may be those described in standard textbooks, such as Chapter 3 of *Protective Groups in Organic Chemistry*, McOmie, Ed., Plenum Press, New York (1973); and Chapter 2 of *Protective Groups in Organic Synthesis*, Greene, John Wiley & Sons, New York (1981).

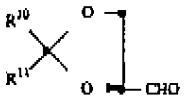
Hydroxy-protecting groups commonly employed include formyl,



2-chloroacetyl, benzyl, diphenylmethyl, triphenylmethyl, 4-nitrobenzyl, phenoxycarbonyl, C_1–C_4 alkyl such as t-butyl, methoxymethyl, tetrahydropyranyl, allyl, tetrahydrothienyl, 2-methoxyethoxymethyl, methoxyacetyl, phenoxyacetyl, isobutyryl, ethoxycarbonyl, and benzyloxycarbonyl. Silyl hydroxy-protecting groups are particularly convenient because most of them are cleaved easily by contact with water or an alcohol. Such groups may include especially trimethylsilyl, as well as isopropyldimethylsilyl, methyldiisopropylsilyl, or triisopropylsilyl. The t-butyldimethylsilyl group is a special case and is preferred as the protecting group in this synthesis; it is more difficult to cleave, requiring a reagent such as a hydrohalic acid to remove it from the hydroxy groups.

Ribose or xylose has a hydroxy group at the 1-position of its ring. In order to react the carbohydrate with the base, to form the compounds employed in this invention, a leaving group must be placed at the 1-position. The leaving groups are those typically used in organic synthesis. The preferred leaving groups are sulfonates, of which the most preferred is methanesulfonate. Other typical leaving groups such as toluenesulfonate, ethanesulfonate, isopropanesulfonate, 4-methoxybenzenesulfonate, 4-nitrobenzenesulfonate, 2-chlorobenzenesulfonate, chloro and bromo also may be used.

The carbohydrates employed in the synthesis of the compounds employed in the present invention are prepared by reacting a D-glyceraldehyde ketonide of the formula

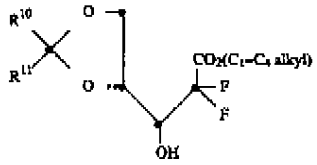


wherein R^{10} and R^{11} are as defined above with a C_1–C_4 alkyl bromodifluoroacetate, preferably the ethyl ester.

The preferred glyceraldehyde ketonide is the acetonide in which R^{10} and R^{11} are both methyl (see Fischer and Baer, *Helv. Chim. Acta*, 17, 622 (1934)). Ethyl bromodifluoroacetate was prepared first by Morel and Dawans, *Tet.* 33, 1445 (1977). The reaction of the ketonide and the haloacetate is carried out in the presence of an activated metal such as magnesium or preferably zinc. Activation is obtained most easily by applying ultrasonic energy to the reaction mixture. Activation by that means compensates for the presence of a small amount of water in the reaction mixture, avoiding the necessity to maintain anhydrous conditions, and also avoids the necessity to prepare and carefully store activated metals. However, if desired, the metal may be activated by the customary methods known in the art. Approximately an equimolar amount of metal is the most advantageous amount.

The reaction has been performed in ethers such as tetrahydrofuran and diethyl ether, at moderate temperatures. However, other organic solvents which are inert to the reaction conditions may be used, including halogenated alkanes such as chloroform, dichloromethane, or trichloroethane, and aromatic solvents including benzene, toluene and the xylenes. Temperatures in the range of from about ambient temperature to about 150° C. may be used; temperatures from about ambient temperature to about 80° C. are preferred, however. Economically-acceptable yields have been obtained in reaction times ranging from a few minutes to a few hours. One should note that the reaction is exothermic, and the mixture may need to be cooled, depending on the scale of the reaction and the rate at which the reactants are added.

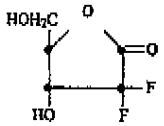
The product of the first reaction is an alkyl 3-dioxolanyl-2,2-difluoro-3-hydroxypropionate of the formula



in which R^{10} and R^{11} are as described above.

The ratio of the 3-R-hydroxy intermediate to its 3-S-hydroxy enantiomer is usually about 3:1. The 3-R-hydroxy enantiomer has the proper stereochemistry to produce the ribose derivative in its natural configuration, and so it is the desired enantiomeric product of the first step. The 3-R-hydroxy enantiomer can generally be separated cleanly from the 3-S-enantiomer by chromatography on silica gel, eluting with chloroform containing 0.5% methanol.

The hydroxypropionate, in either form, is hydrolyzed using very mild conditions to form the lactone of the formula



Proper control of the hydrolysis step will cleave the ketonide function and the ester group, providing the lactone in a single step. The hydrolysis reagent preferably is a mildly acidic ion exchange resin, of which Dowex 50W-X12 (Dow Chemical Company) is most highly preferred. Other mild hydrolytic reagents may be employed although larger amounts of by-products may be obtained. For example, aqueous acetic acid, or other relatively strong acids such as propionic acid, formic acid, chloroacetic acid, or oxalic acid may be used for the hydrolysis.

The hydroxy groups of the lactone should be protected before its keto oxygen is reduced. The usual reaction conditions are used, depending on the protecting groups chosen. For example, the t-butyldimethylsilyl group is most conve-

niently provided in the form of its trifluoromethanesulfonate, and the protection reaction is carried out in the presence of a base such as lutidine, pyridine and the like. Acyl protecting groups such as acetyl, benzoyl and the like are added by reacting the lactone with an acylating agent such as an acyl chloride, bromide, cyanide or azide, or with an appropriate anhydride. The reactions are conveniently carried out in a basic solvent such as pyridine, quinoline or isoquinoline, or in a tertiary amine solvent such as triethylamine, tributylamine, or methylpiperidine. The reaction also may be carried out in an inert solvent, to which an acid scavenger, such as a tertiary amine, has been added. Acylation catalysts such as 4-dimethylaminopyridine or 4-pyrrolidinopyridine may be used in the reaction, if desired. The acylation reactions which provide protecting groups on the hydroxy groups are carried out at moderate temperatures in the range of from −25° C. to 100° C. Such acylations also may be performed by acid-catalyzed reactions of the appropriate carboxylic acids, in inert organic solvents or neat. Acid catalysts such as sulfuric acid, polyphosphoric acid, or methanesulfonic acid may be used.

Acyl protecting groups may also be provided by forming an active ester of the appropriate acid, for example esters formed by reaction with reagents such as dicyclohexylcarbodiimide, acylimidazoles, nitrophenols, pentachlorophenol, N-hydroxysuccinimide and 1-hydroxybenzotriazole.

Protected groups of the ether type are produced by reacting the lactone with, for example, an appropriate diazo compound, such as diazomethane, phenyldiazomethane or a silyldiazomethane. Such reactions commonly are carried out in solvents including esters such as ethyl acetate, halogenated solvents including dichloromethane and chloroform, and ethers including diethyl ether and tetrahydrofuran. The process is usually carried out at low temperatures from about −50° C. to about 0° C. Such ether-forming reactions may also be carried out with the assistance of reagents such as trimethyloxosulfonium hydroxide, trimethylsulfonium hydroxide and trimethylselenonium hydroxide, in solvents such as dimethylsulfoxide, dimethylformamide, hexamethylphosphoramide, acetone, or acetonitrile.

The silyl protecting groups discussed above are placed on the hydroxy groups by the conventional methods, such as by reaction with the appropriate silylcarboxamide or bis(substituted-silyl)carboxamide, or an appropriately substituted silazane. Suitably substituted silyl methanesulfonates, toluenesulfonates and the like are useful also. An equivalent amount of a base is usually necessary in the reaction mixture, unless a basic solvent is used in the reaction.

When the hydroxy groups have been protected, the keto oxygen of the lactone is reduced to the alcohol, forming the protected 2-desoxy-2,2-difluororibose or xylose. The most preferred reducing agent is diisobutyl aluminum hydride, used at a low temperature in the range of about −100° C. to −20° C. The reduction must be performed very carefully to avoid conditions so vigorous that the ring is opened at the oxygen atom. Other metal hydrides, such as the widely used lithium aluminum hydride, can also be used for the reduction, but it is necessary to keep the temperature quite low and to assure that the hydride is destroyed before the temperature is allowed to rise above about −20° C. Accordingly, a solvent with a very low freezing point, such as toluene, must be used in the reduction step. Other solvents, of course, can be used, including lower alkanols, especially ethanol, or ethers such as diethyl ether.

To obtain efficient reaction with the base, an appropriate leaving group must be placed at the 1-position of the carbohydrate. The preferred leaving group is methanesulfonyl, and the compound with this leaving group is readily provided by reaction with methanesulfonyl chloride in the presence of an equivalent amount of a suitable acid scavenger such as triethylamine and the like. Other sulfonyl leaving groups are provided in the same way by reaction with the appropriate sulfonyl halide.

When a chloro or bromo leaving group is to be used, it is frequently advantageous first to make the 1-acetate derivative, as by reaction with acetic anhydride, or another source of acetyl groups, in the presence of an equivalent amount or more of an acid scavenger. The acetate group then is displaced, at a low temperature such as about −50° C. to about 0° C., with gaseous hydrogen bromide or hydrogen chloride. Because the gaseous hydrogen halide may tend to remove the protecting groups, especially silyl protecting groups, operating this step at low temperatures and adding the hydrogen halide slowly in small increments is necessary.

The compounds employed in the present invention having a base portion which is composed of a purine substrate are preferably synthesized by reacting the 1-hydroxy analog of the carbohydrate having protecting groups at the 3- and 5-position with the base in the presence of diethyl azodicarboxylate and triphenylphosphine. Standard modifications are then made to the purine substrate if desired.

The bases used to form the compounds employed in the present invention are known to those skilled in the art, and no discussion of their synthesis is necessary. The primary amino groups present on some of the bases, however, should be protected before the base is coupled with the carbohydrate. The usual amino-protecting groups are employed, including silyl groups such as have been discussed, as well as such typical groups as t-butoxycarbonyl, benzyloxycarbonyl, 4-methoxybenzyluxycarbonyl, 4-nitrobenzyloxycarbonyl, formyl, or acetyl.

Converting the keto oxygen atoms on the bases to the enol form, in order to make the base more highly aromatic and allowing a more ready attack of the base by the carbohydrate is advisable. Enolization is provided most conveniently by producing the silyl protecting groups. The usual silyl protecting groups, as discussed above, may be used for this purpose.

The reaction between the protected carbohydrate and the base preferably is performed neat at a temperature in the range of from about 50° C. to about 200° C. Use of relatively high-boiling solvents for the reaction, such as dimethylformamide, dimethylacetamide, or hexamethylphosphoramide, however, is possible. If the coupling reaction is carried out at elevated pressures to avoid distillation of a low-boiling solvent, any convenient inert reaction solvent can be used.

The coupling reaction may be done at low temperatures if a reaction initiator, such as a trifluoromethanesulfonyloxysilane, is used. The usual inert reaction solvents, as discussed above, may be used at temperatures in the range of from about ambient temperature to about 100° C.

The final step of the reaction sequence is the removal of the protecting groups. Most silyl protecting groups are cleaved easily by contact with water or an alcohol. The t-butyldimethylsilyl protecting group requires acid conditions, such as contact with gaseous hydrogen halide, for its removal.

Acyl protecting groups are removed by simple hydrolysis with strong or moderately strong bases, such as alkali metal hydroxides, at temperatures from about ambient temperature to about 100° C. At least one equivalent amount of base is needed for each protecting group. Such hydrolyses conveniently are carried out in hydroxylic solvents, especially aqueous alkanols. The reactions also may be carried out,

however, in any convenient solvent, such as polyols including ethylene glycol, ethers such as tetrahydrofuran, ketones such as acetone and methyl ethyl ketone and other polar solvents such as dimethylsulfoxide. The cleavage of acyl protecting groups may also be performed with other bases, including, for example, sodium methoxide, potassium t-butoxide, hydrazine, hydroxylamine, ammonia, alkali metal amides and secondary amines such as diethylamine. The acyl protecting groups also can be removed with acid catalysts, such as methanesulfonic acid, hydrochloric acid, hydrobromic acid, sulfuric acid, or with acidic ion exchange resins. Carrying out such hydrolyses at a relatively high temperature, such as the reflux temperature of the mixture is preferred, but temperatures as low as ambient may be used when particularly strong acids are used.

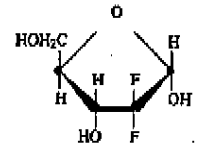
The removal of protecting groups which are ethers is carried out by known methods, for example, with ethanethiol and aluminum chloride.

Compounds of the invention possessing hydroxy or amino acyl or alkyl groups can, of course, be either selectively deprotected, or such groups may be removed and selectively replaced by standard conditions.

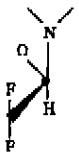
None of the reaction steps require unusual excesses of the reactants. As usual in organic syntheses, use of a moderate excess, in the range of 1.05× to 2×, is advisable.

The compounds employed in this invention are capable of forming pharmaceutically-acceptable addition salts. Such salts are to be construed as included within the scope of this invention and may include hydrobromide, hydrochloride, mono-, di- or triphosphate esters and sodium salts of such phosphates, sulfate, the sodium, potassium, lithium or ammonium salts, as well as others well-known to those skilled in the art. "Pharmaceutically-acceptable salts" are those salts useful in the chemotherapy of warm-blooded animals.

The structural drawings defining the compounds employed in the present invention do not indicate their stereochemistry. Compounds of all configurations are believed to be useful, and the stereochemistry of the compound is not to be construed as a limitation. The preferred compounds possess the configuration of naturally occurring ribose, e.g.,



The configuration at the juncture between the ribose and the base is preferably as follows:



One skilled in the art would be aware of the bases which are used in the synthesis of the nucleosides employed in the present invention, but the following specific nucleosides are given to further elaborate the type of agents which may be used in this invention.

1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-5-chloro-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-5-bromo-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-5-iodo-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose
1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-5-fluoro-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose
1-(4-amino-5-chloro-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose
1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose
1-(4-amino-5-fluoro-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose
1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose
1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluoroxylose
1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluoroxylose

or the pharmaceutically-acceptable salts thereof.

The following Examples illustrate specific compounds suitable for use in the present invention. The Examples are not intended to be limiting in any respect to the scope of the invention and should not be so construed.

EXAMPLE 1

1-(4-Amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

To 47.3 g (0.1 mol) of 3,5-bis(t-butyldimethylsiloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose in 940 ml of dry 1,2-dichloroethane was added 48.0 g (0.16 mol) of bis-trimethylsilyl N-acetylcytosine followed by 39.23 g (0.177 mol) of trifluoromethanesulfonyloxytrimethylsilane. The reaction mixture was refluxed under a nitrogen atmosphere for about 15 hours, cooled to room temperature, and diluted by the addition of 16 ml of methanol. The resulting mixture was stirred for 30 minutes, concentrated under vacuum to about one-half the original volume and cooled in ice. The precipitated solid was collected by filtration and the filtrate was shaken one time with about 300 ml of 10% sodium bicarbonate and one time with brine. The organic layer was separated and concentrated to dryness in vacuo at 45° C. The residue was dissolved into 1.3 l. of methanol saturated with ammonia and the resulting solution was stirred overnight. The volatiles were removed in vacuo at 45° C. to provide 32 g of residue. The residue was dissolved into 275 ml of methanol and 100 g of Biorad cation exchange resin (AG50WX8) was added to the resulting solution. The suspension was stirred at ambient temperature overnight. The resin was removed by filtration and rinsed one time with 100 ml of methanol. The filtrate was discarded and the resin was suspended in 100 ml of methanol and 50 ml of concentrated ammonium hydroxide. This mixture was stirred vigorously for 15 minutes and the resin was filtered. This procedure was repeated two times with additional fresh methanolic ammonia. The basic methanolic filtrates were

combined and evaporated at 45° C. in vacuo to yield a brown foam weighing 13.8 grams. This material was chromatographed with the use of a Waters Prep 500 C_{18} reverse phase column with 100% water to yield 1.26 g of 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose.

NMR (CD_3OD, 90 mHz, δ) 3.7–4.65 (m, 4H), 4.83 (s, 4H), 5.97 (d, J=8 Hz, 1H), 6.24 (t, J=7 Hz, 1H, 7.88 (d, J=8 Hz, 1H). Mass spec. m/e=263=P

EXAMPLE 2

1-(4-Amino-5-iodo-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

To 1.99 g (0.0042 mol) of 3,5-bis(t-butyldimethylsiloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose in 35 ml of dry 1,2-dichloroethane was added 2.08 g (0.0046 mol) of tris-trimethylsilyl-5-iodocytosine followed by 1.11 g (0.005 mol) of trifluoromethanesulfonyloxytrimethylsilane. The reaction mixture was refluxed for about 16 hours under a nitrogen atmosphere and cooled to room temperature. Five milliliters of methanol were added to the reaction mixture and the mixture was stirred for an additional 30 minutes. The mixture was filtered and the precipitated solid was collected by filtration. The filtrate was evaporated to dryness under reduced pressure, and the resulting residue was dissolved in 20 ml of dichloromethane saturated with anhydrous hydrogen bromide. This mixture was stirred for about 3 hours. The volatiles were removed in vacuo at 45° C. The residue was dissolved in 15 ml of water, neutralized to pH 7–8 with 10% sodium bicarbonate, and the resulting solution was washed once with 10 ml of ethyl acetate. The aqueous layer was chromatographed on a Whatman Prep ODS-3 reverse phase column in 2.0 ml portions using water/methanol (9:1, v:v) to afford 30 mg of 1-(4-amino-5-iodo-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose.

NMR (CD_3OD, 90 mHz, δ) 3.47–4.66 (m, 4H), 4.78 (s, 4H), 6.14 (t, J=7 Hz, 1H), 8.32 (s, 1H). Mass spec. m/e=389=P

EXAMPLE 3

1-(2,4-Dioxo-1H, 3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

A solution of 190 mg (0.0007 mol) of 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose in 16 ml of glacial acetic acid and 4 ml of water was refluxed for approximately 24 hours. The reaction mixture was cooled to ambient temperature and the volatiles were evaporated under vacuum at about 60°–70° C. The residue was stirred with 5.0 ml of toluene and the resulting solution was evaporated several times. The residue was dissolved in 12 ml of methanol, and the resulting mixture was cooled to −15° C. and saturated with anhydrous ammonia. The solution was stirred overnight at ambient temperature. The volatiles were removed in vacuo at 45° C. The residue was suspended in about 5.0 ml of hot water and the insoluble material was removed by filtration. The filtrate was chromatographed on a Whatman 50 cm partisil ODS-3 reverse phase column using water methanol (9:1, v:v) as the eluent to afford 0.05 g of product containing a small trace of unreacted starting material. The unreacted starting material was removed by passing a solution of 0.05 g of the mixture in about 5.0 ml of a solvent solution of methylene chloride/methanol (9:1, v:v) through a Waters Silica Sep-Pak. The eluate was evaporated in vacuo at 45° C. to yield 0.036 g of 1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose.

NMR (CD_3OD, 90 mHz, δ) 3.54–4.48 (m, 4H), 4.83 (s, 3H), 5.69 (d, J=8 Hz, 1H), 6.10 (dd, J=7 Hz, 9 Hz, 1H), 7.8 (d, J=8 Hz, 1H). Mass spec. m/e=264=P

EXAMPLE 4

1-(4-Amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose

A solution of 1.86 g (0.0039 mol) of 3,5-bis(t-butylidimethylsilyloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluororibose, 1.87 g (0.0055 mol) of bis-trimethylsilyl-5-methylcytosine and 1.34 g (0.006 mol) of trifluoromethanesulfonyloxytrimethylsilane in 37 ml of dry methylene chloride was refluxed overnight. The reaction mixture was cooled to room temperature and 1.0 ml of methanol was added thereto. The precipitated solid was collected by filtration and the filtrate was evaporated in vacuo at 45° C. The residue was dissolved in 20 ml of water and the resulting solution was concentrated to about 10 ml in vacuo at 50° C. at which point a precipitate formed. The precipitated solid was collected by filtration and the filtrate was concentrated in vacuo at 50° C. to afford 2.2 g of residue. The residue was triturated several times with 10 ml portions of warm acetone. The decanted organic layers were combined and evaporated in vacuo at 45° C. to provide 1.67 g of a yellow oil. This material was dissolved into 15 ml of methanol/water (v:v, 1:1) and the resulting solution was stirred overnight with 5.0 g of Biorad AG50WX8. The suspension was saturated with anhydrous ammonia and stirred for 10 minutes. The resin was collected by filtration and suspended in 30 ml of methanol/ammonia (v:v, 2:1). The solution was stirred for 10 minutes. The resin was collected by vacuum filtration, and the basic filtrates were combined and concentrated in vacuo at 50° C. to provide 1.5 g of an orange oil. The oil was dissolved in 10 ml of water and chromatographed 2.0 ml per run on a Whatman partisil ODS-3 50 cm reverse phase prep column using water as the eluent to provide 0.07 g of 1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose.

NMR (CD_3OD, 90 mHz, δ) 1.94 (s, 3H), 3.53–4.62 (m, 4H), 4.75 (s, 4H), 6.17 (t, J=8 Hz, 1H, 7.67 (s, 1H). Mass spec. m/e=277=P

EXAMPLE 5

1-(4-Amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose

Under a nitrogen atmosphere, to 17.89 g (0.0375 mol) of 3,5-bis(t-butyldimethylsiloxy)-1-methanesulfonyloxy-2-desoxy-2,2-difluoroxylose in 300 ml of dry methylene chloride was added 23.0 g (0.063 mol) of tris-trimethylcytosine followed by 10.84 g (0.0488 mol) of trifluoromethanesulfonyloxytrimethylsilane. The solution was refluxed overnight and cooled to room temperature. Twenty milliliters of methanol were added to the reaction mixture and the resulting solution was stirred vigorously for about one hour. The precipitated solid was collected by filtration. The filtrate was charged with 100 ml of water and the suspension was stirred vigorously for 30 minutes. The organic layer was separated and concentrated in vacuo at 45° C. to give 11.2 g of a brown oil. The oil was dissolved in 95 ml of methanol to which 33 g of Biorad AG5OWX8 cation exchange resin had been added and the suspension was stirred overnight at ambient

temperature. The resin was collected by filtration and washed with 50 ml of methanol. The resin was stirred vigorously with 100 ml of a solution of methanol/ammonia (v:v, 1:1). The resin was collected by filtration and again stirred in this solution. The resin was collected and the basic filtrates were combined and concentrated in vacuo at 50° C. to give 2.09 g of a yellow residue. This material was suspended in 25 ml of water and stirred vigorously for 15 minutes. The insoluble precipitate was filtered to yield 0.250 g of a compound labeled A. The filtrate was concentrated in vacuo at 50° C. to yield 0.86 g of a compound labeled B. Compound A was dissolved in 20 ml of methanol and stirred for 3 days with Biorad AG5OWX8 at ambient temperature. The resin was collected by filtration and slurried in 30 ml of a solution of methanol/concentrated ammonium hydroxide (v:v, 1:1). The resin was collected by filtration and the filtrate concentrated in vacuo at 50° C. to give 0.14 g of 1-(2-desoxy-2,2-difluoro-β-D-xylofuranosyl)cytosine.

NMR (CD_3OD, 90 mHz, δ) 3.72–4.34 (m, 4H), 4.78 (s, 4H), 5.86 (d, J=8 Hz, 1H), 6.17 (d, J=15 Hz, 1H), 7.78 (d, J=8 Hz, 1H). Mass.spec. m/e=263=P

The compound labeled B was chromatographed on a Whatman 50 cm ODS-3 reverse phase prep column using water/methanol (v:v, 1:1) as the eluent to afford 0.06 g of 1-(2-desoxy-2,2-difluoro-α-D-xylofuranosyl)cytosine.

NMR (CD_3OD, 90 mHz, δ) 3.53–3.9 (m, 2H), 4.1–4.57 (m, 2H) 4.83 (s, 4H), 5.9 (d, J=8 Hz, 1H), 6.3 (dd, J=7 Hz, 12 Hz, 1H) 7.55 (d, J=8 Hz, 1H). Mass spec. m/e=263=P

EXAMPLE 6

1-(6-Amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose

A. 1-(6-Chloro-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)- 2-desoxy-2,2-difluororibose

To a solution of 0.77 g (5.0 mmol) of 6-chloro-purine in 50 ml of tetrahydrofuran was added 1.31 g (5.0 mmol) of triphenylphosphine and 0.87 g (5.0 mmol) of diethyl azodicarboxylate. To this solution was added a solution of 1.99 g (5.0 mmol) of 3,5-bis(t-butyldimethylsiloxy)- 1-hydroxy-2-desoxy-2,2-difluororibose in tetrahydrofuran. The reaction mixture was stirred at room temperature for approximately 60 hours and an additional 0.66 g (1.7 mmol) of 3,5-bis(t-butyldimethylsiloxy)-1-hydroxy-2-desoxy-2,2-difluororibose was added to the reaction mixture. The mixture was stirred for an additional 6 hours at room temperature. The solvent was evaporated under vacuum and the residue was stirred in a small amount of diethyl ether overnight. The precipitated solid was removed by vacuum filtration and the filtrate was concentrated under vacuum to dryness. The residue was chromatographed over 70 g of silica and eluted with chloroform. Fractions containing the major component were combined and the solvent was evaporated therefrom to provide 1.0 g of 1-(6-chloro-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)-2-desoxy-2,2-difluororibose. The structure of the product was verified by NMR. Mass spec.=477 [534-(t-butyl)]

B. 1-(6-Amino-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)- 2-desoxy-2,2-difluororibose

A solution of 0.5 g (0,936 mmol) of 1-(6-chloro-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)-2-desoxy-2,2-difluororibose dissolved in 75 ml of absolute ethanol was saturated with anhydrous ammonia at about 0° C. The reaction flask was sealed, and the mixture was allowed to warm to room temperature. The mixture was stirred for about 72 hours at room temperature and the volatiles were evaporated under reduced pressure to provide 420 mg of 1-(6-amino-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)-2-desoxy-2,2-difluororibose. Mass spec=458 [515-(t-butyl)]

C. A solution of 100 mg (0.194 mmol) of 1-(6-amino-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)-2-desoxy-2,2-difluororibose dissolved in 25 ml of methylene chloride cooled to about 0° C. with an external ice bath was saturated with anhydrous hydrogen bromide gas. The mixture was stirred at about 0° C. for about 4 hours, and nitrogen was bubbled through the reaction mixture. The mixture was filtered and the collected solid was washed with methanol to provide 110 mg of solid. The solid was purified by HPLC to provide 12.1 mg of β-1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose and 6.3 mg of α-1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose.

NMR for β-isomer. NMR (CD_3OD, 30 mHz, δ), 3.8–4.65 (m, 4H); 4.83 (bs, 4H); 6.33 (dd, 1H); 8.22 (s, 1H); 8.4 (s, 1H). mass spec. m/e=287

EXAMPLE 7

A. 1-(2,6-Dichloro-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)- 2-desoxy-2,2-difluororibose

To a solution of 1.89 g (10.0 mmol) of 2,6-dichloropurine in 100 ml of tetrahydrofuran was added 2.62 g (10.0 mmol) of triphenylphosphine and 1.74 g (10.0 mmol) of diethyl azodicarboxylate. To this mixture was added a solution of 3.98 g (10.0 mmol) of 3,5-bis(t-butyldimethylsiloxy)-1-hydroxy-2-desoxy-2,2-difluororibose in 25 ml of tetrahydrofuran and the mixture was stirred at room temperature overnight. The precipitated solid was removed by vacuum filtration and the filtrate was concentrated under vacuum. The residue was dissolved in 100 ml of diethyl ether and the solution was stirred at room temperature overnight. The mixture was filtered and the filtrate was evaporated to dryness under vacuum. The residue was dissolved in 25 ml of ethyl acetate, and the mixture was set in the refrigerator. The mixture was filtered and the filtrate was chromatographed by HPLC while eluting with hexane/ethyl acetate (4/1, v/v). The first chromaphore containing fractions were combined and the solvent was evaporated therefrom to provide 2.5 g of 1-(2,6-dichloro-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)-2-desoxy-2,2-difluororibose. m/e=[568-(t-butyl)]=511

B. 1-(2-Chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose and 1-(2-chloro-6-bromo-9H-purin- 9-yl)-2-desoxy-2,2-difluororibose.

A solution of 0.5 g (0.88 mmol) of 1-(2,6-dichloro-9H-purin-9-yl)-3,5-bis(t-butyldimethylsiloxy)-2-desoxy-2,2-difluororibose dissolved in 100 ml of methylene chloride cooled to about 0° C. was saturated with anhydrous hydrogen bromide gas. The mixture was stirred at 0° C. for about 7 hours and then at room temperature for about 16 hours. The mixture was filtered, and the precipitated solid was dissolved in methanol. The methanolic solution was concentration under vacuum to provide 160 mg of a mixture of 1-(2-chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose and 1-(2-chloro-6-bromo-9H-purin-9-yl)-2-desoxy-2,2-difluororibose as a light yellow solid. m/e=322 and 386 respectively.

C. 1-(2-Chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose

A mixture of 1.18 g (3 mmol) of 1-(2-chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose and 1-(2-chloro-6-bromo-9H-purin-9-yl)-2-desoxy-2,2-difluororibose dissolved in 11 ml of 1.0N sodium hydroxide was

stirred at room temperature for three hours. The pH of the mixture was lowered to about 7 with 2N hydrochloric acid. The mixture was concentrated under vacuum at about 45° C. The residue was slurried in warm methanol, filtered and this procedure was repeated. The filtrates were combined and the solution was concentrated under vacuum at 15° C. to provide 1.36 g of 1-(2-chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose. m/e=322.

D. This is the preferred synthesis of 1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose. However, the material prepared by the following reaction was not biologically evaluated, but rather was used as a reference standard for the subsequent synthesis of the compound which was biologically evaluated.

To a suspension of 1.3 g of 1-(2-chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose in 30 ml of absolute ethanol at a temperature of about 0° C. was added anhydrous ammonia. The mixture was placed in a closed reaction vessel and heated at about 150° C. overnight. The mixture was cooled and the solid was collected. The filtrate was suspended in 15 ml of hot methanol and the mixture was again filtered. The filtrate was concentrated under vacuum and the residue was chromatographed by HPLC using water methanol (9/1, v/v) as the eluent at a flow rate of 4 ml/minute to provide 10 mg of α-1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose and 5 mg of β-1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose. m/e=303

The compounds which were biologically tested were prepared as follows:

To 0.26 g of a mixture of 1-(2-chloro-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose and 1-(2-chloro-6-bromo-9H-purin-9-yl)-2-desoxy-2,2-difluororibose in 10 ml of absolute ethanol at about 0° C. was added anhydrous ammonia for 20 minutes. The flask was sealed and placed in an oil bath at about 150° C. for about 16 hours. The volatiles were evaporated under reduced pressure and the residue was purified by standard procedures to provide 9.6 mg of α-1-(2-chloro-6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose having m/e=322; 8.2 mg of β-1-(2-chloro-6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose having m/e=322 and an NMR (CD_3OD, 300 mHz, δ) 3.8–4.65 (m, 4H); 4.93 (bs, 4H); 6.25 (dd, 1H); 8.35 (s, 1H); 6.5 mg of a mixture of α- and β-1-(2,6-diamino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose having (m+1)e=304 and m/e calc. 303.1017; obs. 303.1009; 9.0 mg of 1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose having (m+H)/e and calc. 304.0857; obs. 304.0857; and NMR (CD_3OD, 300 mHz, δ) 3.85–4.65 (m, 4H); 4.9 (bs, 5H); 6.15 (dd, 1H); 7.98 (s, 1H); and 9.0 mg of α- and β-1-(2,6-dioxo-1H,3H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose having m/e=304.

The present invention provides a method of treating susceptible neoplasms in mammals comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of formula I. The method comprises administering the compound to the mammal by various routes including the oral, rectal, transdermal, subcutaneous, intravenous, intramuscular or intranasal routes.

The term "pharmaceutically effective amount", as defined herein, refers to an appropriate amount of a compound of formula I which is capable of providing chemotherapy to mammals. The active compounds are effective over a wide dosage range. For example, dosages per day will normally fall within the range of about 0.1 to about 1200 mg/kg of body weight. In the treatment of adult humans, the range of about 0.1 to about 50 mg/kg, in single or divided doses, is preferred. However, it will be understood that the amount of compound actually administered will be determined by a physician, in the light of the relevant circumstances including the condition to be treated, the particular compound to be administered, the chosen route of administration, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way.

The term "susceptible neoplasm", as defined herein, represents an abnormal growth of tissue in mammals capable of being treated by a compound of formula I. While the compounds of formula I are effective against tumors, both solid and non-solid type, the compounds are effective in controlling the growth of rapidly dividing cells because of the compounds' cytotoxic nature. It is a special feature of these compounds that they have a broad spectrum of activity, and are accordingly useful against a variety of tumors.

The compounds of the present method are preferably administered as a pharmaceutical formulation. Therefore, as yet another embodiment of the present invention, a pharmaceutical formulation useful for treating susceptible neoplasms in mammals is provided comprising a compound of formula I with a pharmaceutical carrier, diluent or excipient therefor.

The active ingredient will be present in the formulation in the range of about 1% to about 90% by weight. The active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained release of the active ingredient after administration to the patient by employing procedures well known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 500 mg, more usually about 25 to about 300 mg, of the active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

The following formulation examples represent specific pharmaceutical formulations employing compounds comprehended by the present method. The formulations may employ as active compounds any of the compounds of

Formula I. The examples are illustrative only and are not intended to limit the scope of the invention in any way.

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose	250
Starch dried	200
Magnesium stearate	10

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

A tablet formula is prepared using the ingredients below:

	Quantity (mg/tablet)
1-(2-oxo-4-amino-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoro-ribose	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

An aerosol solution is prepared containing the following components:

	Weight %
1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluoro-ribose	0.25
Ethanol	29.75
Propellant 22 (Chlorodifluoromethane)	70.00

The active compound is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to −30° C. and transferred to a filling device. The required amount is then placed in a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

Tablets each containing 60 mg of active ingredient are made up as follows:

1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoro-ribose	60 mg
Starch	45 mg
Microcrystalline cellulose	35 mg
Polyvinylpyrrolidone (as 10% solution in water)	4 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1 mg

The difluoronucleoside starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°–60° C. and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

Capsules each containing 80 mg of medicament are made as follows:

1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluor-oxyloss	80 mg
Starch	59 mg
Microcrystalline cellulose	59 mg
Magnesium stearate	2 mg

The active ingredient, cellulose, starch and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

Suppositories each containing 225 mg of nucleoside are made as follows:

1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluoro-ribose	225 mg
Saturated fatty acid glycerides to	2 g

The nucleoside is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

Formulation 7

Suspensions each containing 50 mg of medicament per 5 ml dose are made as follows:

1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose	50 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 ml
Benzoic acid solution	0.10 ml
Flavor	q.v.

-continued

Color	q.v.
Purified water to	5 ml

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation 8

An intravenous formulation is prepared as follows:

1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoro ribose	100 mg
Isotonic saline	1000 ml

The solution of the above ingredients is administered intravenously at a rate of 1 ml/minute to a mammal in need of treatment from susceptible neoplasms.

The activity of representative compounds employed in the present invention has been demonstrated in standard screens commonly used by those in the art in testing compounds for potential antitumor drugs. For example, these screens have been used to demonstrate the antitumor activity of commercially available cancer drugs such as the vinca alkaloids. See, e.g., Miller et al., in *J. Med. Chem.* Vol. 20, No. 3 409 (1977) and Sweeney, et al., in *Cancer Research* 38, 2886 (1978).

The compounds represented by formula I employed in the present invention are cytostatic in that they inhibit the growth of human leukemic cells (CCRF-CEM cell line). Table 1 below gives the results of such testing of several compounds representative of those in Formula I. In the Table, column 1 gives the name of the compound and column 2 the IC_{50} (concentration giving 50% growth inhibition) in mcg/ml.

TABLE 1

Cytotoxicity Screen

Compound Name	IC_{50} mcg/ml
1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose	0.0039 0.0057 0.0068

TABLE 1-continued

Cytotoxicity Screen

Compound Name	IC_{50} mcg/ml
	0.0260
1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose	0.3
1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose	5.4
1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose	0.3
β-1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose	0.5
α-1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose	6.9
α-1-(2-chloro-6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose	>20.0
β-1-(2-chloro-6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose	0.4
1-(2,6-diamino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose	0.075
1-(2-amino-6-oxo-1H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose	0.10
1-(2,6-dioxo-1H,3H,9H-purin-9-yl)-2-desoxy-2,2-difluororibose	0.30

To further demonstrate the ability of the compounds of formula I to treat susceptible neoplasms in mammals, the compounds of Example 1, 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose, Example 5, 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose, and Example 6, 1-(6-amino-9H-purin-9-yl)-2-desoxy-2,2-difluororibose, were tested in animals bearing a tumor system representative of L1210V lymphocytic leukemia.

The study testing the efficacy of these compounds against L1210V leukemia was initiated by an IP inoculation of 1×10^6 cells. Treatment was begun 24 hours after inoculation. The response to therapy was determined by comparing the mean life span of the ten treated animals to that of the ten control animals; prolongation of life in the treated animals beyond that of controls is expressed as a percentage. Table 2 gives the results of several experiments in mice bearing this tumor. In the Table, column 1 gives the example number of the compound tested; column 2, the experiment number; column 3, the dose level of the compound in mg/kg; column 4, the route of administration; column 5, the dosage schedule, that is, the days on which the compound was administered to the mice; column 6, the average increase in life span of the treated mice as compared to the control mice; column 7, the toxic deaths over the number of mice in each group; and column 8, the indefinite survivors, that is, the number of 45 day survivors in each group.

TABLE 2

L1210V Lymphocytic Leukemia Tumor System

Example No. of Compound Tested	Experiment No.	Dose Level mg/kg	Route of Administration	Dosage Schedule	Percent Increase Life Span	Toxic Deaths	Indefinite Survivors
1	1	20.0	IP	Days	60	0/10	0
		10.0		1,5,9	66	1/10	0
		5.0			66	0/10	0
		2.5			60	0/10	0
		1.25			50	0/10	0
	2	2.0	IP	Daily	0	0/10	0

TABLE 2-continued

Example No. of Compound Tested	Experiment No.	Dose Level mg/kg	Route of Administration	Dosage Schedule	Percent Increase Life Span	Toxic Deaths	Indefinite Survivors
					L1210V Lymphocytic Leukemia Tumor System		
		1.0		for 10	0	0/10	0
		0.5		Days	13	0/10	0
	3	200.0	IP	Day 1	34	0/10	0
		100.0		only	26	0/10	0
		50.0			30	0/10	0
		25.0			23	0/10	0
	4	4.0	PO	Daily	2	4/10	0
		2.0		for 10	18	1/10	0
		1.0		days	15	0/10	0
		0.5			8	0/10	0
	5	4.0	IP	Daily	44	0/10	0
		2.0		for 9	136	0/10	0
		1.0		days	104	0/10	0
		0.5			74	0/10	0
5	1	30.0	IP	Daily for 10 days	57	0/10	0
6	1	200.0	IP	Daily	18	0/7	0
		100.0		for 9 days	16	0/7	0

The compounds of Example 1, 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose, and Example 5, 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose, also demonstrated activity in additional tumor test systems. These systems include the 6C3HED lymphosarcoma, also known as the Gardner lymphosarcoma (6C3HED); the CA-755 adenocarcinoma (CA755); the P1534J lymphatic leukemia (P1534J); and the X5563 plasma cell myeloma (X5563). Each of these systems is described in detail below.

6C3HED—The 6C3HED lymphosarcoma was obtained in 1979 from the Division of Cancer Treatment, N.C.I., tumor bank maintained at E.G. and G. Mason Research (Worchester, Mass.). First passage tumor was stored in liquid nitrogen using standard techniques. The transplanted tumor was re-established from the tumor bank every six months or as needed. The tumor is maintained by serial passage twice weekly in C3H mice (Charles River; Wilmington, Mass.).

CA755—The adenocarcinoma 755 is an undifferentiated mammary carcinoma which was obtained in 1980 from the Division of Cancer Treatment, N.C.I., tumor bank maintained at E.G. and G. Mason Research (Worchester, Mass.). First passage tumor was stored in liquid nitrogen using standard techniques. The transplanted tumor was re-established from the tumor bank every six months or as needed. The tumor is maintained by serial passage once a week in C57BL/6 female mice (Jackson Laboratory; Bar Harbor, Me.).

P1534J—The P1534J lymphocytic leukemia (solid form) was obtained in 1973 from the Jackson Laboratory (Bar Harbor, Me.). First passage tumor was stored in liquid nitrogen using standard techniques. Subsequent replenishment of the tumor bank with this tumor was accomplished from first passage tumor. The transplanted tumor was re-established from the tumor bank every six months or as needed. The tumor is maintained by serial passage once a week in DBA/2 mice (Charles River; Wilmington, Mass.).

X5563 Mycloma—the tumor is maintained in C3H mice.

The following procedure was employed in demonstrating the activity of these compounds against the tumor systems. The tumor was removed from passage animals and minced into 1 to 3 mm square fragments using sterile techniques. Tumor pieces were checked for sterility using both Antibiotic Medium 1 and Brain Heart Infusion (Difco; Detroit, Mich.). Recipient mice were shaved and tumor pieces were implanted subcutaneously in the axillary region by trocar. Drug therapy on the appropriate schedule was initiated on the day after tumor implant. The compound was dissolved in saline for all experiments. All animals were weighed at the beginning and end of drug treatment. Food and water were provided ad libitum. On days 10 to 12, two dimensional measurements (width and length) of all tumors were taken using vernier calipers. Tumor weights were calculated from these measurements using the following formula:

$$\text{Tumor Weight (mg)} = \text{Tumor Length (mm)} \times \text{Tumor Width (mm)}^2/2$$

For all data, the tumor weight was rounded to the nearest tenth of a gram for analysis. No group is included in the analysis for therapeutic activity in which deaths attributable to drug toxicity exceeded 30 percent of the treated group.

In Table 3 which follows, column 1 gives the example number of the compound tested; column 2 provides the tumor system; column 3, the dose level; column 4, the route administered; column 5, the dosage schedule; column 6, the percent inhibition of the tumor; and column 7, the toxic deaths observed prior to completion of the study.

TABLE 3

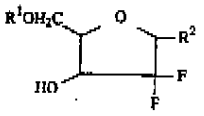
Activity of Example 1 Against A Variety of Tumor Models

Example No. of Compound Tested	Tumor System	Dose Level mg/kg	Route of Administration	Dosage Schedule	Percent Inhibition of Tumor	Toxic Deaths
1	6C3HED	20.0	IP	Days 1,5,9	95	0/7
		10.0			88	0/7
		5.0			49	0/7
		2.5			1	0/7
		1.25			0	0/7
	6C3HED	0.4	IP	Daily for 8 Days	8	0/10
		0.2			0	0/10
		0.1			0	0/10
		0.05			0	0/10
	CA755	20.0	IP	Days 1,5,9	94	0/10
		10.0			86	0/10
		5.0			85	0/10
		2.5			44	0/10
		1.25			0	0/10
	P1534J	20.0	IP	Days 1,5,9	92	1/10
		10.0			71	2/10
		5.0			47	0/10
		2.5			30	0/10
		1.25			8	0/10
	X5563	20.0	IP	Days 1,5,9	100	0/10
		10.0			98	0/10
		5.0			89	0/10
		2.5			52	1/10
		1.25			11	1/10
5	X5563	30.0	IP	Daily for 9 days	28	0/10

The compounds employed in the present method are also effective for the treatment of viral infections, and more particularly in the treatment of infections caused by viruses of the herpes genus. The compounds are effectively administered orally, topically or parenterally. In general, dosage rates in the range of from about 5 mg/kg to about 500 mg/kg are useful. It is more preferred to administer at rates in the range of from about 10 mg/kg to about 100 mg/kg.

We claim:

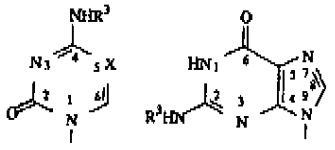
1. A method of treating susceptible neoplasms in mammals comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of the formula



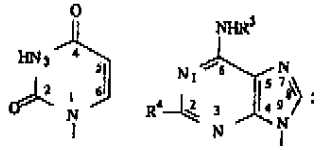
wherein:

R^1 is hydrogen;

R^2 is a base defined by one of the formulae



-continued



X is C–R^4;

R^3 is hydrogen;

R^4 is hydrogen, C_1–C_4 alkyl, bromo, fluoro, chloro or iodo;

and the pharmaceutically-acceptable salts thereof.

2. The method of claim 1 in which the compound is 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose or a pharmaceutically acceptable salt thereof.

3. The method of claim 1 in which the compound is 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluoroxylose or a pharmaceutically acceptable salt thereof.

4. The method of claim 1 in which the compound is 1-(2,4-dioxo-1H,3H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose or a pharmaceutically acceptable salt thereof.

5. The method of claim 1 in which the compound is 1-(4-amino-5-methyl-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose or a pharmaceutically acceptable salt thereof.

6. The method of claim 1 wherein the susceptible neoplasm is selected from the group consisting of leukemias, sarcomas, carcinomas, and myelomas.

7. The method of claim 6 employing 1-(4-amino-2-oxo-1H-pyrimidin-1-yl)-2-desoxy-2,2-difluororibose or a pharmaceutically acceptable salt thereof.

* * * * *

CIVIL COVER SHEET

County in which this action arose: Wayne

...civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as ...d by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose ...initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS
SUN PHARMACEUTICAL INDUSTRIES LTD.

(b) County of Residence of First Listed Plaintiff: Mumbai, India
(EXCEPT IN U.S. PLAINTIFF CASES)

(c) Attorney's (Firm Name, Address, and Telephone Number)
Raymond M. Kethledge (P49235) and Moheeb H. Murray (P63893)
Bush Seyferth Kethledge & Paige PLLC, 3001 W. Big Beaver Rd., Ste. 600, Troy, MI 48084 (248) 822-7800

DEFENDANTS
ELI LILLY AND COMPANY

County of Residence of First Listed Defendant
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE LAND INVOLVED.

Attorneys (If Known)

II. BASIS OF JURISDICTION (Select One Box Only)

- ☐ 1 U.S. Government Plaintiff
- ☒ 3 Federal Question (U.S. Government Not a Party)
- ☐ 2 U.S. Government Defendant
- ☐ 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES

	(Plaintiff) PTF	(Defendant) DEF
	1 ☐ 4	☐ 4
	5 ☐	☐ 5
	6 ☐	☐ 6

Case: 2:07-cv-15087
Judge: Steeh, George Caram
Referral MJ: Whalen, R. Steven
Filed: 11-29-2007 At 04:12 PM
CMP SUN PHARMACEUTICAL V ELI LILLY AND COMPANY (RRH)

IV. NATURE OF SUIT (Select One Box Only)

CONTRACT
- ☐ 110 Insurance
- ☐ 120 Marine
- ☐ 130 Miller Act
- ☐ 140 Negotiable Instrument
- ☐ 150 Recovery of Overpayment & Enforcement of Judgment
- ☐ 151 Medicare Act
- ☐ 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- ☐ 153 Recovery of Overpayment of Veteran's Benefits
- ☐ 160 Stockholders' Suits
- ☐ 190 Other Contract
- ☐ 195 Contract Product Liability
- ☐ 196 Franchise

REAL PROPERTY
- ☐ 210 Land Condemnation
- ☐ 220 Foreclosure
- ☐ 230 Rent Lease & Ejectment
- ☐ 240 Torts to Land
- ☐ 245 Tort Product Liability
- ☐ 290 All Other Real Property

TORTS

PERSONAL INJURY
- ☐ 310 Airplane
- ☐ 315 Airplane Product Liability
- ☐ 320 Assault, Libel & Slander
- ☐ 330 Federal Employers' Liability
- ☐ 340 Marine
- ☐ 345 Marine Product Liability
- ☐ 350 Motor Vehicle
- ☐ 355 Motor Vehicle Product Liability
- ☐ 360 Other Personal Injury

PERSONAL INJURY
- ☐ 362 Personal Injury - Med. Malpractice
- ☐ 365 Personal Injury - Product Liability
- ☐ 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- ☐ 370 Other Fraud
- ☐ 371 Truth in Lending
- ☐ 380 Other Personal Property Damage
- ☐ 385 Property Damage Product Liability

CIVIL RIGHTS
- ☐ 441 Voting
- ☐ 442 Employment
- ☐ 443 Housing/ Accommodations
- ☐ 444 Welfare
- ☐ 445 Amer. w/Disabilities - Employment
- ☐ 446 Amer. w/Disabilities - Other
- ☐ 440 Other Civil Rights

PRISONER PETITIONS
- ☐ 510 Motions to Vacate Sentence

Habeas Corpus:
- ☐ 530 General
- ☐ 535 Death Penalty
- ☐ 540 Mandamus & Other
- ☐ 550 Civil Rights
- ☐ 555 Prison Condition

FORFEITURE/PENALTY
- ☐ 610 Agriculture
- ☐ 620 Other Food & Drug
- ☐ 625 Drug Related Seizure of Property 21 USC 881
- ☐ 630 Liquor Laws
- ☐ 640 R.R. & Truck
- ☐ 650 Airline Regs.
- ☐ 660 Occupational Safety/Health
- ☐ 690 Other

LABOR
- ☐ 710 Fair Labor Standards Act
- ☐ 720 Labor/Mgmt. Relations
- ☐ 730 Labor/Mgmt.Reporting & Disclosure Act
- ☐ 740 Railway Labor Act
- ☐ 790 Other Labor Litigation
- ☐ 791 Empl. Ret. Inc. Security Act

BANKRUPTCY
- ☐ 422 Appeal 28 USC 158
- ☐ 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- ☐ 820 Copyrights
- ☒ 830 Patent
- ☐ 840 Trademark

SOCIAL SECURITY
- ☐ 861 HIA (1395ff)
- ☐ 862 Black Lung (923)
- ☐ 863 DIWC/DIWW (405(g))
- ☐ 864 SSID Title XVI
- ☐ 865 RSI (405(g))

FEDERAL TAX SUITS
- ☐ 870 Taxes (U.S. Plaintiff or Defendant)
- ☐ 871 IRS—Third Party 26 USC 7609

OTHER STATUTES
- ☐ 400 State Reapportionment
- ☐ 410 Antitrust
- ☐ 430 Banks and Banking
- ☐ 450 Commerce
- ☐ 460 Deportation
- ☐ 470 Racketeer Influenced and Corrupt Organizations
- ☐ 480 Consumer Credit
- ☐ 490 Cable/Sat TV
- ☐ 810 Selective Service
- ☐ 850 Securities/Commodities/ Exchange
- ☐ 875 Customer Challenge 12 USC 3410
- ☐ 890 Other Statutory Actions
- ☐ 891 Agricultural Acts
- ☐ 892 Economic Stabilization Act
- ☐ 893 Environmental Matters
- ☐ 894 Energy Allocation Act
- ☐ 895 Freedom of Information Act
- ☐ 900 Appeal of Fee Determination Under Access to Justice
- ☐ 950 Constitutionality of State Statutes

V. ORIGIN (Select One Box Only)

☒ 1 Original Proceeding ☐ 2 Removed from State Court ☐ 3 Remanded from Appellate Court ☐ 4 Reinstated or Reopened ☐ 5 Transferred from another district (specify) ☐ 6 Multidistrict Litigation ☐ 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION
Cite the U.S. Civil Statute under which you are filing (Do not cite jurisdictional statutes unless diversity):
28 U.S.C. §§ 2201, 2202 and 21 U.S.C. 355§
Brief description of cause:
Declaratory judgment of patent invalidity and non-infringement

VII. REQUESTED IN COMPLAINT:
☐ CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23
DEMAND $
CHECK YES only if demanded in complaint:
JURY DEMAND: ☐ Yes ☒ No

VIII. RELATED CASE(S) IF ANY (See instructions): JUDGE _____ DOCKET NUMBER _____

DATE: November 29, 2007
SIGNATURE OF ATTORNEY OF RECORD: Raymond M. Kethledge (P49235)

FOR OFFICE USE ONLY

RECEIPT # _____ AMOUNT _____ APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____

SUANT TO LOCAL RULE 83.11

1. Is this a case that has been previously dismissed?

☐ Yes
☒ No

If yes, give the following information:

Court: _____

Case No.: _____

Judge: _____

2. Other than stated above, are there any pending or previously discontinued or dismissed companion cases in this or any other court, including state court? (Companion cases are matters in which it appears substantially similar evidence will be offered or the same or related parties are present and the cases arise out of the same transaction or occurrence.)

☒ Yes
☐ No

If yes, give the following information:

Court: Southern District of Indiana

Case No.: 1:06-cv-1721-SEB-JMS

Judge: Sarah Evans Barker

Notes :